PATENT APPLICATION

Our Docket No. 20050022.ORI

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re App	:	Colin M. Casimir	: October 14, 2009
S.N.	:	10/520,745	: Art Unit 1632
Filed	:	August 22, 2005	:Examiner Wu Cheng Winston Shen
For	:	METHODS OF MAKING VIRAL PARTICLES HAVING A MODIFIED CELL BINDING ACTIVITY AND USES THEREOF	

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I CERTIFY THAT THIS PAPER IS BEING TRANSMITTED VIA THE OFFICE ELECTRONIC FILING SYSTEM ADDRESSED TO: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450, ON OCTOBER 14, 2009 (37 CFR 1.8a)

Barbara L Davis

Sir:

Colin M. Casimir declares that:

1. He is the inventor of the subject matter of the above-captioned patent application and he is familiar with the details of the contents of that application, including the present claims.

2. He is familiar with the Official Action received from the United States Patent and Trademark Office dated April 14, 2009, and the present enablement issue and understands the position of the Examiner to be that the application is enabling only insofar as it relates to the expression of hCSF on a retroviral packaging cell and the targeting of a c-kit-expressing cell.

3. He is familiar with the general state of the art regarding the making of retroviral particles having modified cell binding activity.

4. He has conducted or supervised the acquisition of additional experimental data that he believes demonstrates that proteins other than hSCF can be expressed on a retroviral packaging cell. A copy of that data is attached to this Declaration as Exhibit A.

5. Considering the results in Exhibit A, he has demonstrated, and one should conclude, that the scope of enablement is commensurate with the claimed subject matter, extending to proteins beyond hSCF and that this has been confirmed by relatively routine testing and without the need for extensive or "undue" experimentation.

6. He further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 14 October , 2009

Colin M. Casimir

Targeted transduction of cells expressing MCSF receptor (c-fms) using virus displaying surface MCSF

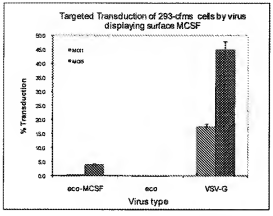


Fig 1. Targeted Transduction of 293-cfms cells by virus displaying surface MCSF

Human 293T fibroblasts modified to express surface c-fms the macrophage colony stimulating factor (MCSF) receptor were transduced, at two different multiplicities of infection, with lentiviruses pseudotyped with a permissive envelope (VSV-G), a non-permissive envelope (eco), or a non-permissive envelope combined with the surface display of the MCSF targeting ligand (eco-MCSF). As expected no transduction was observed with an ecotropic pseudotyped virus but significant levels of transduction (that increased with increasing moi) were obtained with the targeted virus

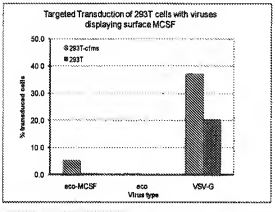


Fig 2a Targeted Transduction of 293-cfms cells by virus displaying surface MCSF

Repeat of experiment described in Fig 1 but comparing the transduction efficiency of unmodified 293T cells (293T) with cell modified to express surface cfms (293T-cfms). Lentivirus pseudotyped with ecotropic envelope (eco) is unable to transduce human cells, however the virus displaying surface MSCF is able to transduce the human cells expressing the cognate receptor for MCSF (293T-cfms) but not unmodified human cells showing that transduction is dependent on the ligand/receptor interaction.

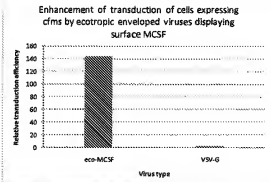


Fig 2b Enhancement of transduction of cells expressing cfms by ecotropic enveloped viruses displaying surface MCSF

Data from Fig 2a showing the increase in transduction efficiency through display of MCSF on the lentiviral surface. With the permissive virus (VSV –G) transduction of 293T-cfms cells and of 293T cells is virtually equivalent (<2X more efficient). In contrast, the targeted virus (eco-MCSF) is able to transduce the cfms-expressing cells >140X more efficiently than unmodified cells.

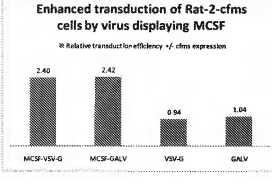
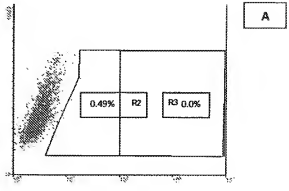
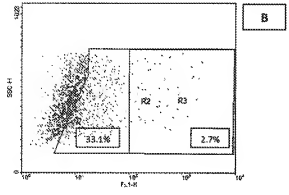


Fig 3. Enhanced transduction of Rat-2-cfms cells by virus displaying MCSF

Rat2 cells or Rat2 cells modified to express surface cfms were transduced with lentiviruses with VSV-G or GALV envelopes (both permissive). Viruses displaying surface MCSF (the ligand for cfms) showed enhanced transduction on cells expressing the cognate receptor whilst those not expressing MCSF showed no enhancement.

293T cells engineered to express human IL-2 on their surface





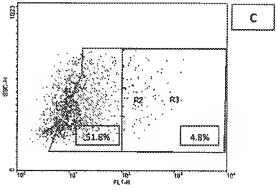


Fig 4. Human IL-2 expression on the surface of 293T cells

293T cells were transfected with an expression plasmid (pREP8) containing a cDNA encoding human IL-2, using the calcium phosphate precipitation method. The complete IL-2 coding region was fused in-frame to a membrane anchor region derived from human SCF. Following transfection the cells were cultured in the presence of histidinol to select for the presence of the IL-2 construct and derive stably transfected cell lines. After culture for 5 weeks the cells were tested for expression of surface IL-2 using a mouse antihuman IL-2 antibody, conjugated with FITC. The results obtained from two different clonal isolates are shown. The proportion of cells expressing detectable IL-2 was ~35% in one case (Fig4b) and 55% in the other (Fig4c). The fluorescence gates were set using untransfected cells also stained with antibody (Fig 4a). Negative cells are shown in red. Positive cells are shown in green and highly positive cells in blue

Targeted transduction of primary human T cells using virus displaying surface IL-2

The IL-2 expressing virus packaging cells shown above (see Fig 4) were used to generate lentivirus particles that display surface IL-2. These viruses were tested for their ability to transduce primary human lymphocytes, separated from whole blood using ficoll-hypaque. T cells, the lymphocyte sub-population that expresses surface IL-2 receptor were identified in the bulk population by staining for CD3, a T cell specific marker. Gene delivery to total primary human lymphocytes (Fig 5A), or to T cells, (Fig 5B) was only successful when the cells were transduced with virus displaying surface IL2. As expected virus expressing only the ecotropic envelope, which is non-permissive for human cells were unable to transduce the primary lymphocytes. Interestingly, the virus pseudotyped with the permissive VSV-G envelope was also unable to transfect the target cells. This was unexpected and suggests that quiescent, or non-activated T cells were refractory to transduction. Only T cells that were activated by engagement of the IL-2 receptor were capable of being transduced, indicating that the surface displayed IL-2 of the targeted virus is biologically active as a cytokine, as well as being able to function in viral transduction.

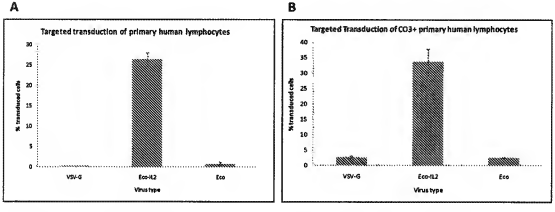


Fig 5 **Targeted transduction of human primary T cells with lentivirus displaying surface IL-2 .**
Human primary lymphocytes isolated from donor blood were transduced with lentiviruses pseudotyped with permissive envelope (VSV-G), non-permissive envelope (Eco), or non-permissive envelope plus targeting (Eco-IL2). A, total lymphocyte population B, CD3+ cells. Data from 2 independent experiments.